

Geographic Variation in Clutch and Egg Size for the Lizard *Phrynocephalus przewalskii* (Squamata: Agamidae)

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Abstract In order to tease apart proximate vs. ultimate sources of variation in reproductive strategy, studies have increasingly focused on populations rather than species as the unit of interest. The reproductive parameters of *Phrynocephalus przewalskii* (Agamidae) in different populations within the same phylogenetic clade were compared in this study. Female SVL, clutch size, egg volume and clutch volume varied significantly among populations. With increase in latitude, clutch size increased, while egg size decreased. Relatively fewer but larger eggs were produced with increasing of population density. Food availability had positive effects on clutch size, but no effect on egg size. Our result indicated that latitude, food availability and population density may be the proximate factors affecting the reproductive parameters of *P. przewalskii*.

Keywords *Phrynocephalus przewalskii*, reproductive parameter, geographic variation, egg size, clutch size

1. Introduction

A key objective in the research of life history evolution is to understand the ecological factors favoring different life history strategies (Partridge and Harvey, 1988). Life history strategies often exhibit considerable variation at different levels: among individuals within the same population (e. g., Ballinger, 1977; Schwarzkopf, 1992; Abell, 1999), among populations of a single species (e. g., Du *et al.*, 2005; Jin and Liu, 2007; Zuffi *et al.*, 2009), and among different species (e. g., Dunham, 1988; Zamora-Abrego *et al.*, 2007). The variation in life history characteristics may arise from several sources, such as genetic divergence caused ultimately by natural selection and phenotypic plasticity induced proximately by environment factors. Teasing apart proximate vs. ultimate sources of the variation in reproductive parameters remains a major challenge for life history studies.

As ectotherms, lizards highly depend upon ambient

climatic conditions and display substantial environmentally induced variation in life history features and, as such, have become ‘model organisms’ in evolutionary ecology (Vitt and Pianka, 1994; Pinaka, 1999). An approach that has become popular in analyses of reptilian life-history evolution is applied and compared among conspecific populations (Ferguson and Talent, 1993; Qualls *et al.*, 1995). Previous studies suggested that the variation in life history strategies of lizards were the adaptive responses to various selective environments, such as altitude (Hancock *et al.*, 1998; Jin and Liu, 2007), latitude (Wilson and Cooke, 2004; Du *et al.*, 2005), food availability (Ballinger, 1977; Abell, 1999; Bonnet *et al.*, 2001) and climate (e. g., precipitation and temperature) (Adolph and Porter, 1996; Zuffi *et al.*, 2009).

Phrynocephalus przewalskii is a small toad-headed oviparous sand lizard which has a large distribution range in northern China and adjacent Mongolia (Urquhart *et al.*, 2009). It has been extensively studied in systematic and ecological aspects (e. g., Xu and Yang, 1995; Liu *et al.*, 1996; Urquhart *et al.*, 2009). However, the patterns of variation in clutch size, egg size and mating behavior in different geographic and climatic zones, and the sources of the variation have not been investigated. The phylogenetic study on *P. przewalskii* with mitochondrial DNA revealed two major lineages, with clear population

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Received: 28 December 2010 Accepted: 13 May 2011

genetic structure within both clades (Urquhart *et al.*, 2009). The clutch size, egg size, and mating behavior, etc. of nine populations from one group in Clade I (Urquhart *et al.*, 2009) were compared in this study. Our main objectives are to provide detailed information of the variation in reproductive traits among populations, and to understand the relationship between environmental factors and reproductive features in *P. przewalskii*.

2. Materials and Methods

Specimens of *P. przewalskii* from nine sites around the Tengger Desert (Figure 1, Table 1) were sampled during the breeding season between April and June in 2008. Specimens were deposited at the Herbarium of Lanzhou University, were euthanized immediately and then fixed in 8% formalin. Before gonadal analyses, body mass and snout-vent length (SVL) of each specimen were measured. The body mass of females was weighed by electronic scales. The SVL of 261 reproductive females from the nine populations were measured by calipers. Clutch size (CS) was estimated from counts of enlarged vitellogenic follicles or oviductal eggs after dissection. A preliminary analysis showed that the estimated CS from counts of vitellogenic follicles and oviductal eggs had no difference (ANCOVA, $P > 0.05$ in all populations).

Therefore, the data were pooled. Length and width of oviductal eggs were measured to the nearest 0.1 mm, and the volume was calculated with the formula for the volume of an ellipsoid: $V = \frac{4}{3}\pi ab^2$, where a is half of the shortest diameter and b is half of the longest diameter.

Insect abundance was surveyed using pitfall traps (20 traps; 20×10×10 cm) placed in the habitat where the specimens were collected. The traps were set for a 24-h period and the mass of insect was weighed to 0.01 g. The population density of *P. przewalskii* was estimated by the number of individuals encountered during a field search which was done between 10:00 and 15:00 hours. Food availability was estimated by dividing insect mass by population density.

All data were ln or arc-sine transformed to achieve the conditions for using parametric tests. The mean value for SVL of females differed among populations and the mean clutch size varied significantly among populations, and both were analyzed by one-way ANOVA. The relationship of clutch size and body length (SVL) was evaluated by linear regression analysis. If linear correlations were not statistically significant, comparisons of inter-population characteristics were tested by one-way ANOVA. Otherwise, the one-way ANCOVA with maternal size as the covariate was used. The distribution of clutch size was different among populations and

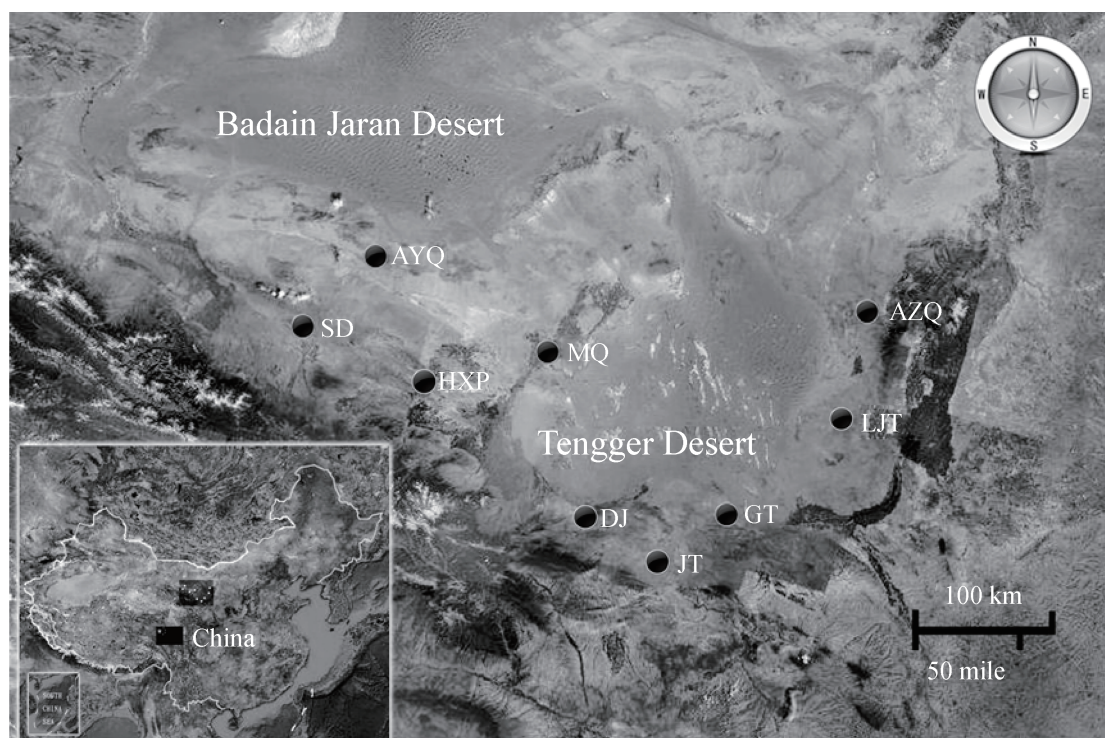


Figure 1 Locations of the populations of *P. przewalskii* examined in this study. Shaded area represents the distribution of this lizard (Urquhart *et al.*, 2009). Alax Zouqi (AZQ); Gantang (GT); Minqin (MQ); Shandan (SD); Alax Youqi (AYQ); Hexipu (HXP); Jingtai (JT); Dajing (DJ); Luanjingtian (LJT).

Table 1 Female reproductive characteristics of *P. przewalskii* in 9 populations within the same phylogenetic clade. All values are mean \pm SE (n: number of observed individuals).

Populations	Altitude (m)	Latitude (°N)	Population density	Food availability	SVL (mm)	Clutch size	n	SVL (mm)	Egg size (mm ³)	n
AZQ	1448	38.85	30	0.231	52.9 \pm 0.6	3.1 \pm 0.1	35	52.5 \pm 1.3	433.3 \pm 17.7	8
GT	1614	37.47	40	0.185	51.7 \pm 0.6	2.4 \pm 0.2	39	50.0 \pm 0.8	408.6 \pm 23.7	13
MQ	1369	38.58	19.2	0.223	50.3 \pm 0.6	2.4 \pm 0.1	38	49.7 \pm 1.3	440.6 \pm 20.5	12
SD	1789	38.78	12	0.339	52.6 \pm 0.9	3.2 \pm 0.2	30	55.2 \pm 1.3	352.0 \pm 33.6	11
AYQ	1491	39.22	12	0.589	51.7 \pm 0.8	3.3 \pm 0.2	34	52.1 \pm 1.1	329.2 \pm 16.2	14
HXP	1704	38.39	5.7	1.644	50.0 \pm 0.8	3.0 \pm 0.1	29	52.1 \pm 0.8	427.6 \pm 28.9	10
JT	1736	37.13	24	0.129	52.7 \pm 1.6	3.1 \pm 0.3	16	55.4 \pm 2.6	425.8 \pm 21.3	7
DJ	1886	37.49	45	0.888	52.3 \pm 0.9	2.4 \pm 0.2	21	51.4 \pm 0.9	427.8 \pm 29.3	10
LJT	1336	37.97	14	0.273	55.2 \pm 0.9	3.4 \pm 0.2	19	51.2 \pm 2.8	400.4 \pm 44.9	4
Total					51.9 \pm 0.3	2.9 \pm 0.1	261	52.0 \pm 0.5	400.9 \pm 9.3	89

was analyzed by Chi-square test. The correlation of clutch size with latitude and food availability, and the negative correlation with population density of different populations were analyzed by partial correlation analysis. Clutch size variation among populations was checked by Chi-square test. Partial correlation analysis was applied to check the correlation of clutch sizes with latitude and food availability, with sample sizes of different populations being controlled. Both clutch size and female SVL were ln-transformed, and then were analyzed by linear regression to determine the effect of female body condition on the relative fecundity. Same statistical method was then used to determine the tradeoff between egg size and relative fecundity. All data were given as mean \pm SE and significance was set as $P < 0.5$. $P < 0.05$ was considered as statistically significant.

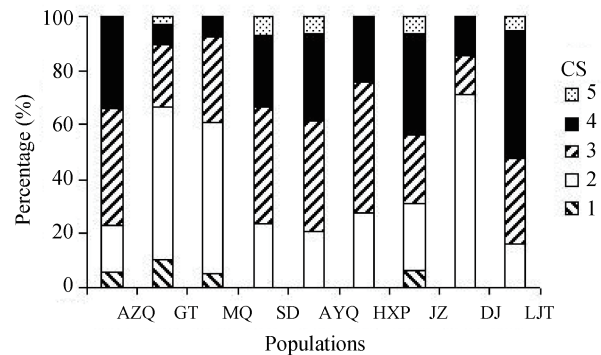
3. Results

3.1 Female body size We measured 261 reproductive females from the nine populations (Table 1), and obtained the mean value of SVL of different populations (ANOVA, $F_{8,252} = 2.97$, $P = 0.003$). Pearson correlation analysis indicated that there was no significant correlation between female SVL and altitude ($r = -0.024$, $P = 0.696$) or latitude ($r = -0.043$, $P = 0.490$).

Linear regression analysis suggested that female SVL had significant effect on the clutch size of eight populations ($P < 0.022$ in all case) except for Minqin ($r = 0.305$, $F_{1,87} = 3.70$, $P = 0.062$), while no significant effect on egg volume was found in any populations ($P > 0.059$ in all case). When the data were pooled, clutch size was positively associated with female body size ($r = 0.493$, $F_{1,259} = 83.30$, $P < 0.001$), while egg volume was not ($r = 0.060$, $F_{1,259} = 0.31$, $P = 0.578$).

3.2 Clutch size Clutch size of *P. przewalskii* ranged from

1 to 6, and mean clutch size varied significantly among populations (ANOVA, $F_{8,252} = 6.37$, $P < 0.001$, Table 1). When the effect of maternal body size was controlled by ANCOVA, the difference in clutch size among populations was still significant (ANCOVA, $F_{8,251} = 6.78$, $P < 0.001$). The distribution of clutch size was different among populations (Chi-square test, $\chi^2_{32} = 70.529$, $df = 32$, $P < 0.001$, Figure 2). The prevalent clutch sizes of the populations, Alax Zouqi (AZQ), Shandan (SD), Alax Youqi (AYQ), Hexipu (HXP), Jingtai (JT), Dajing (DJ) and Luanjintan (LJT) were 2 to 4, while Minqin (MQ) and Gantang (GT) were 2 and 3 (Figure 2).

**Figure 2** The distribution of clutch size

Partial correlation analysis indicated that clutch size was positively correlated with latitude ($r = 0.265$, $P < 0.000$, Figure 3) and food availability ($r = 0.157$, $P = 0.011$), respectively, and negatively correlated with population density ($r = -0.355$, $P < 0.001$), but it was not correlated with altitude ($r = -0.023$, $P = 0.708$).

89 female specimens which contained oviductal eggs were used in egg and clutch volume analysis. Significant population variation in the mean egg volume (ANOVA, $F_{8,80} = 3.19$, $P = 0.003$) and clutch volume (ANCOVA, $F_{8,79} = 2.05$, $P = 0.051$) showed similar trend. Pearson

correlation analysis indicated that egg volume was positively correlated with population density ($r = 0.217$, $P = 0.041$), and negatively correlated with latitude ($r = -0.276$, $P = 0.009$, Figure 3), but it was not correlated with altitude ($r = -0.028$, $P = 0.792$) and food availability ($r = 0.020$, $P = 0.854$). Clutch volume showed a trend of negative correlation with population density ($r = -0.206$, $P = 0.054$), but it was not correlated with other factors ($P > 0.160$ in all case).

3.3 Trade-off between egg size and relative fecundity

No significant relationship between egg volume and relative fecundity was found in any population ($P > 0.065$ in all case). When the data were pooled, a trend of negative correlation was found between these two traits ($r = -0.204$, $F_{1,87} = 3.78$, $P = 0.055$). The coefficient of the variation in clutch size (ranging from 24.5% to 40.5%) was higher than that of egg volume (ranging from 11.5% to 22.4%) for eight populations, except for SD population (27.6% vs. 31.7%). The overall coefficient of variance of the mean of the population clutch size was also larger than that of egg volume (32.4% vs. 21.8%).

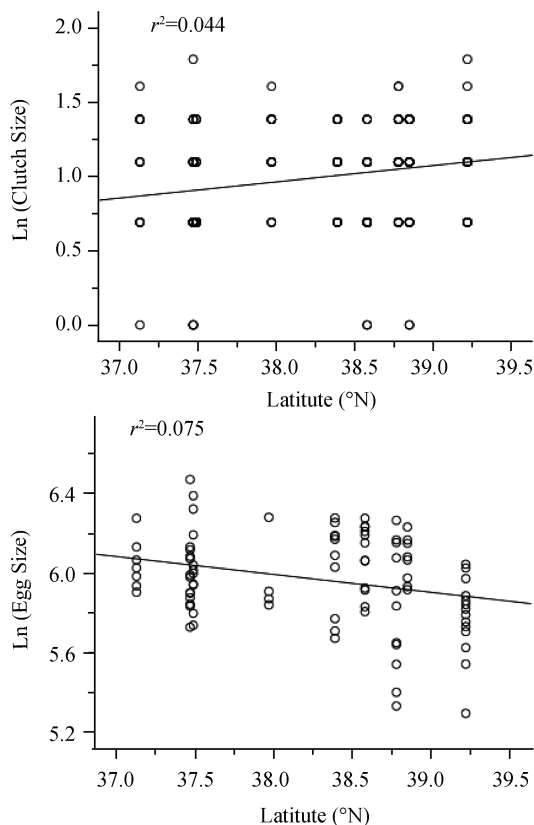


Figure 3 Effect of latitude gradients on clutch size and egg size

4. Discussion

A significant positive correlation was found between

female SVL and clutch size in *P. przewalskii*. Such a relationship has been revealed common in other lizard species with different clutch sizes (e. g., Du *et al.*, 2005; Jin and Liu, 2007). But the female SVL could not explain all the variations in clutch size, because the difference was still significant after the female SVL was statistically controlled. The usual clutch size was different among populations, and the distribution of the clutch size varied significantly among populations.

We failed to find any significant correlation between female SVL and egg volume in almost all the populations. The lack of correlation between female SVL and egg volume suggested that egg volume might be optimized by local adaptation. Our conclusion was further proven by the fact that the clutch size of *P. przewalskii* was more variable than its egg size which was in accordance with the prediction from the optimality models (which is based on the assumption that at any point in an organism's life history there is an optimum percentage of available energy that should be diverted to reproduction to maximize the parents' total contribution to future generations; Smith and Fretwell, 1974). Significant geographic variation of mean egg volume was found, which might be restricted to a specific population.

High altitude characterized by short warm period, low mean temperature and great fluctuation in food availability (e. g., Kovshar, 1981) has profound effects on the life history strategies of lizards (e. g., Badyaev, 1997). Indeed, the species inhabiting high altitudes increase investment per offspring through producing small clutches of large eggs (Hancock *et al.*, 1998; Jin and Liu, 2007) in order to increase the survival of their offspring. However, no correlation was found between altitude and clutch size or egg volume in *P. przewalskii*. This might be due to the altitude gradient being too small to detect any differences.

Latitudinal effect upon reproductive traits was another focused issue (e. g., Wilson and Cooke, 2004; Du *et al.*, 2005). Clutch size usually increased with increasing latitude, while egg size decreased (e. g., Dunham, 1988). *P. przewalskii* inhabiting higher latitudes also produced relatively more but smaller eggs. Most hypotheses formulated to explain this pattern fall into two broad categories: the reproduction-survival tradeoff and the "lack" clutch hypotheses (e. g., Cooper *et al.*, 2005). The first is the 'reproduction-survival tradeoff hypothesis', in which females that allocate more energy toward survival must allocate less toward reproduction (e. g., Moreau, 1944). The second category is the 'lack clutch hypothesis', which assumes that clutch sizes are constrained by the amount of food parents could bring to

the brood (Ashmole, 1963; Cody, 1966; Royama, 1969). It is known that with the increasing latitude, predation decreases, while the overwinter mortality increases. Lizards residing at high latitude undergo considerable mortality in winter, and breed during the spring when food is also plentiful, while those inhabiting at low latitudes undergo heavier predation. In order to achieve high reproductive success, lizards produce relatively fewer but larger offsprings at low latitude, but relatively more but smaller offsprings at high latitude.

Population density can be used as an indicator of the intensity of intraspecific competition to some extent. Under crowded conditions, lizards exhibit unique life history characteristics, such as small clutch size, delayed maturity and low frequency of egg production (Case, 1983). It was known that larger offspring had a survival advantage under the conditions of increased predation and competition. When the intensity of intraspecific competition was high, *P. przewalskii* produced relatively fewer but larger eggs and increased investment per offspring by decreasing the total investment. Through this transformation of the reproductive strategy, the female's total fitness might be enhanced by increasing the survival of offsprings.

Low food availability for reptiles could result in the reduction in clutch size (e. g., Ballinger, 1977; Agrawal *et al.*, 2001) and even delay in reproduction. We found an increased clutch size and clutch mass with increasing food availability, while egg volume did not vary significantly. The result from this study suggested that the females of *P. przewalskii* under good conditions would increase reproductive investment through increasing the number instead of the quality of their offspring.

In summary, *P. przewalskii* clutch size increased with increasing of latitude, while egg size decreased. Relatively fewer but larger eggs were produced with increasing of population density. Food availability had positive effect on clutch size, but no effect on egg size. This study was restricted within the same phylogenetic clade, even within the same population group. So, we can say that latitude, food availability and population density may be the proximate factors which affect the reproductive characters of *P. przewalskii*.

Acknowledgements We are grateful to the National Climate Data Center for providing us the historical climatic data, and to Di WU and Ying WANG from Lanzhou University for helping in collection and measurement of the specimens used in this study.

References

- Abell A. J.** 1999. Variation in clutch size and offspring size relative to environmental conditions in the lizard *Sceoporus virgatus*. *J Herpetol*, 33(2): 173–180
- Adolph S. C., Porter W. P.** 1996. Growth, seasonality, and lizard life histories: Age and size at maturity. *Oikos*, 77: 267–278
- Agrawal A. F., Brodie E. D., Brown J.** 2001. Parent-offspring coadaptation and the dual genetic control of maternal care. *Science*, 292: 1710–1712
- Ashmole N. P.** 1963. The regulation of numbers of tropical oceanic birds. *Ibis*, 103: 458–473
- Badyaev A. V.** 1997. Avian life history variation along altitudinal gradients: An example with *Cardueline finches*. *Oecologia*, 111: 365–374
- Ballinger R. E.** 1977. Reproductive strategies: Food availability as a source of proximal variation in a lizard. *Ecology*, 58: 628–635
- Bonnet X., Naulleau G., Shine R., Lourdaïs O.** 2001. Short-term versus long-term effects of food intake on reproductive output in a viviparous snake, *Vipera aspis*. *Oikos*, 92: 297–308
- Case T. J.** 1983. Niche overlap and the assembly of island lizard communities. *Oikos*, 41: 427–433
- Cody M. L.** 1966. A general theory of clutch size. *Evolution*, 20: 174–184
- Cooper C. B., Hochahka W. M., Butcher G., Dhondt A. A.** 2005. Seasonal and latitudinal trends in clutch size: Thermal constraints during laying and incubation. *Ecology*, 86: 2018–2031
- Du W. G., Ji X., Zhang Y. P., Xu X. F., Shine R.** 2005. Identifying sources of variation in reproductive and life-history traits among five populations of a Chinese lizard (*Takydromus septentrionalis*, Lacertidae). *Biol J Linn Soc*, 85: 443–453
- Dunham A. E., Miles D. B., Resnick D. N.** 1988. Life history pattern in Squamate reptiles. In Gans G., Pough F. H. (Eds), *Biology of the Reptilia*. New York: Academic Press, 441–522
- Ferguson G. W., Talent L. G.** 1993. Life-history traits of the lizard *Sceloporus undulatus* from two populations raised in a common laboratory environment. *Oecologia*, 93: 88–94
- Hancock M. A., Hughes J. M., Bunn S. E.** 1998. Influence of genetic and environmental factors on egg and clutch sizes among populations of *Paratya australiensis* Kemp (Decapoda: Atyidae) in upland rainforest streams, southeast Queensland. *Oecologia*, 115: 483–491
- Jin Y. T., Liu N. F.** 2007. Altitudinal variation in reproductive strategy of the toad-headed lizard, *Phrynocephalus vlangalii* in North Tibet Plateau (Qinghai). *Amphibia-Reptilia*, 28: 509–515
- Kovshar A. F.** 1981. Peculiarities of the bird reproduction insubalpine: On the material of Passeriformes in the Tien Shan. Alma-Ata: Academy of the Kazakh SSR
- Liu N. F., Chen Q., Xie X. M.** 1996. Reproductive ecology of *Phrynocephalus przewalskii*. *Acta Ecol Sin*, 16: 276–282 (In Chinese)

- Moreau R. E.** 1944. Clutch size: A comparative study, with reference to African birds. *Ibis*, 86: 286–347
- Patridge L., Harvey P. H.** 1988. The ecological context of life history evolution. *Science*, 241: 1439–1445
- Pianka E. R.** 1999. *Evolutionary Ecology* (6th Edition). California: Benjamin Cummings
- Qualls C. P., Shine R., Donnellan S., Hutchinson M.** 1995. The evolution of viviparity within the Australian scincid lizard *Lerista bougainvillii*. *J Zool Lond*, 237: 13–26
- Royama T.** 1969. A model for global variation of clutch size in birds. *Oikos*, 20: 562–567
- Schwarzkopf L.** 1992. Annual variation of litter size and offspring size in a viviparous skink. *Herpetologica*, 48(4): 390–395
- Smith C. C., Fretwell S. D.** 1974. The optimal balance between size and number of offspring. *Am Nat*, 108: 499–506
- Urquhart J., Wang Y. Z., Fu J. Z.** 2009. Historical vicariance and male-mediated gene flow in the toad-headed lizards *Phrynocephalus przewalskii*. *Mol Ecol*, 18: 3714–3729
- Vitt L. J., Pianka E. R.** 1994. *Lizard Ecology: Historical and Experimental Perspectives*. Princeton: Princeton University Press
- Wilson B., Cooke D. E.** 2004. Latitudinal variation in rates of overwinter mortality in the lizard *Uta stansburiana*. *Ecology*, 85: 3406–3417
- Xu H. G., Yang F. X.** 1995. Simulation model of activity of *Phrynocephalus przewalskii*. *Ecol Model*, 77: 197–204
- Zamora-Abrego J. G., Zúñiga-Vega J. J., Nieto-Montes de Oca A.** 2007. Variation in reproductive traits within the lizard genus *Xenosaurus*. *J Herpetol*, 41(4): 630–637
- Zuffi M. A. L., Gentili A., Cecchinelli E., Pupin F., Bonnet X., Filippi E., Luiselli L. M., Barbanera F., Dini F., Fasola M.** 2009. Geographic variation of body size and reproductive patterns in continental versus Mediterranean asp vipers, *Vipera aspis*. *Biol J Linn Soc*, 96: 383–391